Functional Coexpression of HSV-1 Thymidine Kinase and Green Fluorescent Protein: Implications for Noninvasive Imaging of Transgene Expression

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Abstract

Current gene therapy technology is limited by the paucity of methodology for determining the location and magnitude of therapeutic transgene expression *in vivo*. We describe and validate a paradigm for monitoring therapeutic transgene expression by noninvasive imaging of the herpes simplex virus type 1 thymidine kinase (HSV-1-*tk*) marker gene expression. To test proportional coexpression of therapeutic and marker genes, a model fusion gene comprising green fluorescent protein (gfp) and HSV-1-*tk* genes was generated (*tkgfp* gene) and assessed for the functional coexpression of the gene product, TKGFP fusion protein, in rat 9L gliosarcoma, RG2 glioma, and W256 carcinoma cells. Analysis of the TKGFP protein demonstrated that it can serve as a therapeutic gene by rendering *tkgfp* transduced cells sensitive to ganciclovir or as a screening marker useful for identifying transduced cells by fluorescence microscopy or fluorescence-activated cell sorting (FACS). TK and GFP activities in the TKGFP fusion protein were similar to corresponding wild-type proteins and accumulation of the HSV-1-*tk*–specific radiolabeled substrate, 2′-fluoro-2′-deoxy-1β-D-arabinofuranosyl-5-iodo-uracil (FIAU), in stability transduced clones correlated with gfp-fluorescence intensity over a wide range of expression levels. The *tkgfp* fusion gene itself may be useful in developing novel cancer gene therapy approaches. Valuable information about the efficiency of gene transfer and expression could be obtained by non-invasive imaging of *tkgfp* expression with FIAU and clinical imaging devices (gamma camera, positron-emission tomography [PET], single photon emission computed tomography [SPECT]), and/or direct visualization of *gfp* expression *in situ* by fluorescence microscopy or endoscopy.

Keywords: thymidine kinase, ganciclovir, FIAU, cancer gene therapy, fusion genes, imaging.

Introduction

Experimental and clinical cancer gene therapy strategies were first developed by using transduction of the thymidine kinase gene of the herpes simplex virus type 1 (HSV-1-*tk*) into proliferating tumor cells [1–5]. Further approaches for gene therapy of cancer have used genes encoding other prodrug activating enzymes, such as cytochrome P450 [6]; cell-cycle regulating proteins, such as p53 and pRB [7]; inhibitors of angiogenesis [8]; or cytokines [9] to enhance the immune response to tumor antigens or have taken advantage of the selective oncolysis mediated by mutant herpes or adenoviruses [10–19] [20,21]. Successful gene therapy of tumors in a clinical setting appears to be limited by insufficient and nonspecific gene delivery. Noninvasive imaging technologies that provide information about the localization, magnitude, and duration of therapeutic transgene expression *in vivo* would greatly facilitate assessment and implementation of novel gene therapy vectors with better gene delivery and expression characteristics.

Currently, several approaches are being developed to establish clinically applicable noninvasive imaging technologies of transgene expression [22–29]. These imaging technologies are based on the detection of marker enzyme–mediated accumulation of specific radiolabeled marker substrates [22–25], binding and accumulation of marker receptor–specific compounds [26–28], or binding and accumulation of gene-specific oligonucleotides [29] with gamma camera, positron emission tomography (PET), and magnetic resonance imaging (MRI).

The vast majority of therapeutic genes, however, do not have appropriate marker substrates that can be radiolabeled or would accumulate in transduced tissues to a level that could be detected by noninvasive imaging. Therefore, it is necessary to develop indirect imaging strategies for monitoring the expression of different therapeutic genes. This can be achieved by functional coexpression of marker and therapeutic genes. The most important requirement of this approach is a proportional and constant coexpression of the marker gene (HSV-1-*tk* or *gfp*) and ''therapeutic'' gene over the wide range of expression levels. Proportionality of coexpression may be achieved by using an internal ribosome entry site (IRES) element of picornaviruses [30] to link the

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two genes in an expression cassette under control of a single promoter [31–35]. A more direct approach, is to produce a fusion gene that would encode for a ''marker/therapeutic'' fusion protein [36,37].

In this report, we describe a paradigm for monitoring the expression of therapeutic transgenes that retain functional activity when coexpressed as a fusion protein. This can be achieved when both the therapeutic and marker subunits of the fusion protein retain functional activity. In this context, we constructed a *tkgfp* fusion gene and assessed the proportionality of HSV-1 thymidine kinase (TK) and green fluorescent protein (GFP) activity over the wide range of expression levels in transduced cells in culture.

Materials and Methods

Cell Culture

Rat 9L gliosarcoma cells [38] were grown as monolayers in Dulbecco's modified Eagle medium (DMEM; Mediatech, Washington, DC) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO) and 100 U/mL penicillin and 100 μg/mL streptomycin (P/S; Sigma). The RG2 and W256 cell lines were grown in MEM supplemented with 10% FBS and antibiotics. All cells were grown at 37°C in a 5% Carbon dioxide/95% air atmosphere.

Cloning of the tkgfp Fusion Gene

The cDNA encoding the thymidine kinase gene of HSV-1 was obtained by digestion of pLTKRNL [2] with BamHI. To facilitate further cloning, the 2.2-kb fragment encoding the open reading frame of the HSV-1-*tk* was isolated and ligated into plasmid, pcDNA3.1/Zeo (Invitrogen), after digestion with BamHI. The resulting plasmid (pTKZeo3.2; 7.8 kb) was sequentially digested with BamHI and XmaI. BamHI cuts 57 base pairs (bp) in front of the start codon of *tk* and XmaI cuts 22 bp in front of the STOP codon. The resulting

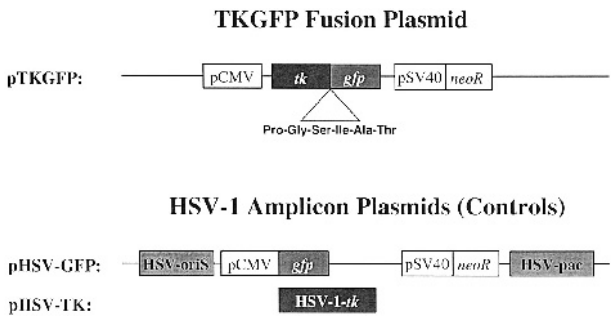

Figure 1. *Structure and elements of TK and GFP expressing plasmids pTKGFP, pHSV-TK (HSV-1 amplicon plasmid as positive control for TK function) and pHSV-GFP (HSV-1 amplicon plasmid as positive control for GFP function). All genes of interest, tkgfp, native HSV-1-tk, and native gfp, respectively, are cloned under control of the CMV immediate early 1 promoter (pCMV). Further structural elements comprise a neomycin resistance gene (neoR) under control of an SV40 promoter. Controls contain also the HSV-1 origin of DNA replication (oriS) and the HSV-1 DNA cleavage/packaging signal (pac), which would allow packaging of these amplicon plamids into HSV-1 virions.*

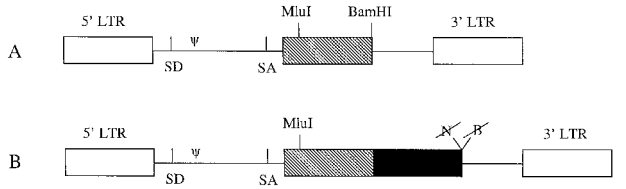

Figure 2. *Construction of the SFG-TKGFP retroviral vector. The vector MoT (**A**) was used to produce the SFG-TKGFP vector (**B**) from pTKGFP (see Figure 1).*

1.2-kb *tk* fragment was isolated and ligated to the 4.7-kb DNA fragment obtained after plasmid pEGFP-N1 (Invitrogen) was digested sequentially with BglII and XmaI. After ligation, the open reading frames of both *tk* and *gfp* are in-frame within the pTKGFP expression plasmid (5.9 kb), placing the *tkgfp* cDNA under control of the cytomegalovirus (CMV) immediate early (IE) 1 promoter (Figure 1).

Cloning of the tkgfp Fusion Gene into a Retroviral Vector

A retroviral vector encoding the fusion protein TKGFP was constructed in the Moloney murine leukemia virus–based SFG vector backbone [39], as previously described [40]. pTKGFP was digested with Not I, then treated with Klenow to blunt the fragment, and then digested with Mlu I. The vector MoT (Figure 2A), which encodes HSV-1-*tk* alone [41], was digested with BamHI, blunted with Klenow, and then digested with Mlu I. The construct was named as SFG-TKGFP (Figure 2B). Ten micrograms of plasmid DNA was transfected by calcium phosphate coprecipitation as previously described [40] into the GPG29 packaging cell line [42]. VSV-G–pseudotyped particles were used as cell free viral stocks [43] to transduce tumor cell lines.

Retroviral Transduction of Tumor Cell Lines

The rat RG2 glioma and rat W256 carcinoma cells were exposed to the medium containing SFG-TKGFP retrovirus ($\sim 10^6$/mL) in presence of polybrene (8 μg/mL) for 8 hours. GFP-positive transduced RG2TKGFP+ and W256TKGFP+ cells were detected by fluorescence microscopy. Single-cell–derived clones were obtained from a mixed population of transduced RG2TKGFP+ and W256 TKGFP+ cells by seeding the cells into the 96 well plates at a density of 0.1 cell/well. GFP-positive RG2TKGFP+ and W256 TKGFP+ colonies were identified by fluorescence microscopy and used for further propagation into single-cell–derived clonal populations.

Functional Assessment of pTKGFP

The initial functional assessment of the *tkgfp* gene construct included the determination of green fluorescence and ganciclovir (GCV)-sensitivity in transiently transfected rat 9L gliosarcoma cells in culture. For both evaluations, 80% confluent monolayers of 9L cells (600,000/35mm dish) were transfected with the plasmid pTKGFP or control plasmids

pHSV-TK and pHSV-GFP (see the next section) or no vector with lipofectamine (GIBCO BRL), according to the manufacturer's protocol (1 μg DNA + 6 μl lipofectamine per 35-mm dish). For fluorescent detection of *gfp*-expressing cells, culture plates were examined with a standard fluorescence microscope (Zeiss IM35; Carl Zeiss Inc, Thornwood, NY) 24 hours after transfection. For determination of the ganciclovir sensitivity, GCV-treatment was carried out at final concentrations of 0, 0.1, 0.3, 1, 3 and 9 μg/mL medium for half of the dishes starting 24 hours after transfection. Four days later, surviving 9L cells were determined as a percentage of non–GCV-treated transfected cells. A *tk*-bearing HSV-1 amplicon plasmid (pHSV-TK, 11.8 kb) featuring the *tk* gene under control of the CMV IE1 promoter (Figure 1) and an HSV-1 amplicon plasmid containing *gfp* under control of the CMV IE1 promoter (pHSV-GFP; 10.4 kb; Figure 1) served as positive controls. All experiments were performed in triplicate and repeated 3 times.

Functional Assessment of GFP Expression in Transduced Clones

The expression of GFP in retrovirally transduced single-cell derived clones of RG2TKGFP+ and W256 TKGFP+ cells was assessed by fluorescence-activated cell sorting (FACS) analysis of GFP fluorescence on a FACSstar Plus analyzer (Becton Dickinson, NJ). Cells were detached by trypsinization, counted suspended in phosphate-buffered solution (PBS) at 4°C at a density of 10^6 cells/mL. GFP fluorescence was measured at the wave length of 488 nm. Parental (nontransduced) RG2 and W256

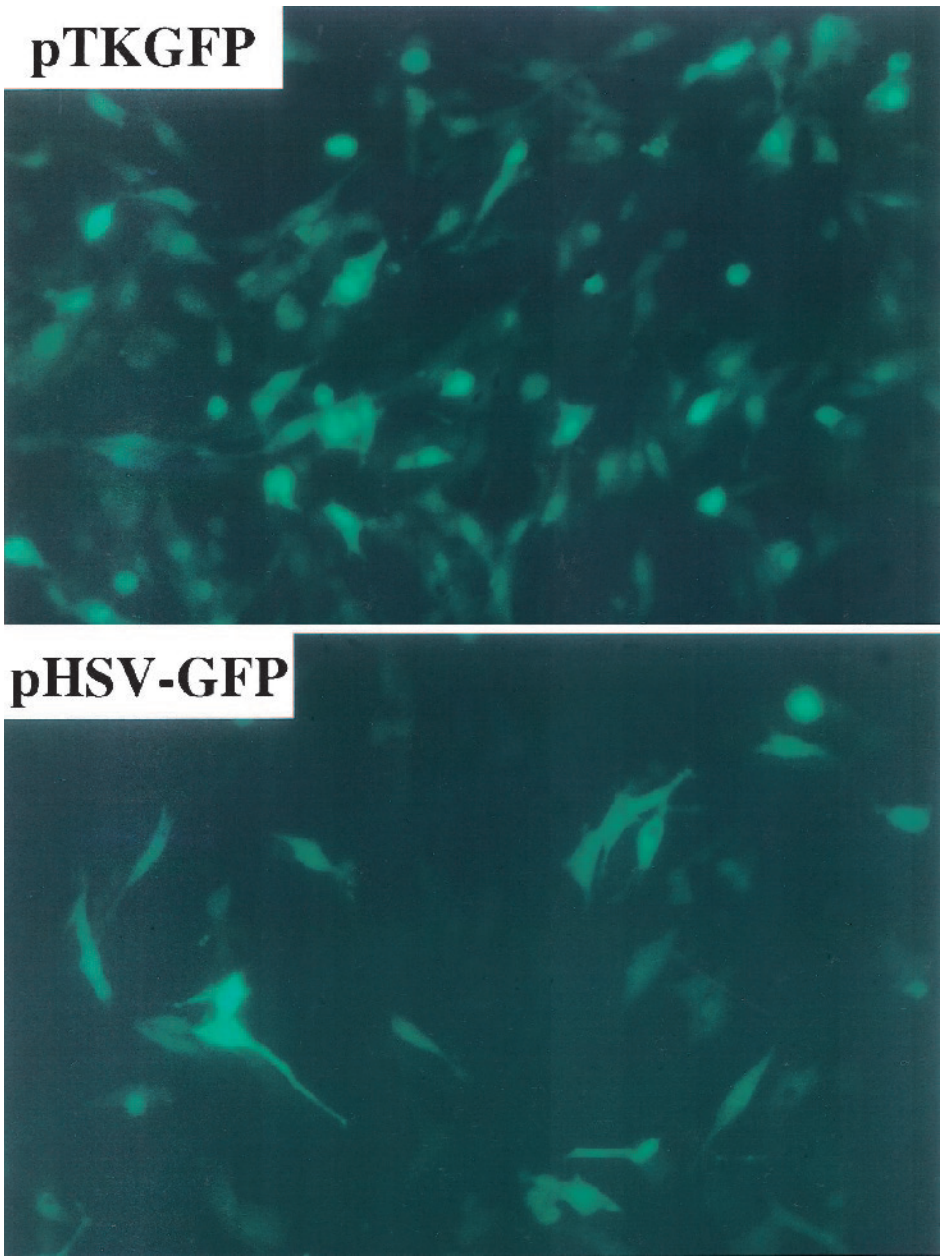

Figure 3. *GFP expression in proliferating rat 9L gliosarcoma cells transfected with pTKGFP or pHSV-GFP. Comparable intensities of gfp fluorescence were identified in 9L cells 24 hours after transfection for both the pTKGFP expression plasmid and the pHSV-GFP amplicon plasmid (positive control).*

cells were used as negative controls. Gates were designed to include viable single cells only. The range of fluorescence was set to include no more than 1% of corresponding control cells. The mean GFP fluorescence per cell was used as a measure of GFP expression in a clonal population of transduced cells.

Functional Assessment of HSV-1-TK Expression in Transduced Clones

The single-cell–derived clones of RG2TKGFP+ and W256 TKGFP+ cells were seeded in 150 × 25-mm culture dishes (at 5000 cells per dish) and grown until 50% confluent. The incubation medium was replaced with 14 mL of medium containing 2-[^{14}C]-FIAU (fialuridine) (56 mCi/mmol) and methyl-[^{3}H]-TdR (65.4 mCi/mmol) (Moravek Biochemicals, Brea, CA). Radiochemical purity of each compound was checked in our laboratory by high performance liquid chromatography (HPLC) and found to be more than 97% pure. The concentrations of 2-[^{14}C]-FIAU and methyl-[^{3}H]-TdR (thymidine deoxyribose) were 0.01 and 0.1 μCi/mL, respectively. The cells were harvested by using a scraper after various periods of incubation (10, 30, 60, 90, and 120 minutes), weighed, and assayed with a Packard B1600 TriCarb β-spectrometer and standard ^{3}H and ^{14}C dual-channel counting techniques. The medium was counted before and after incubation. The data were expressed as a harvested cell-to-medium concentration ratio (dpm/g cells)/(dpm/mL medium) and plotted against time [22]. The steady-state accumulation rate of FIAU, normalized by that of TdR, was obtained from the slopes of the plot and used as a measure of HSV-1-*tk* gene expression in culture (FIAU/TdR ratio).

Western Blot Analysis

The expression of TK and GFP were evaluated by Western blotting with a rabbit polyclonal HSV-TK antiserum and a rabbit anti-GFP antibody (CLONTECH, Palo Alto, CA). Twenty-four hours after transfection of 4 μg each of purified pTKGFP, pHSV-TK (positive control for TK), or pHSV-GFP (positive control for GFP) into 9L cells, monolayers were harvested and resuspended in phosphate buffered saline (1 × PBS) and processed as described previously [44]. In brief, after cell lysis, samples were boiled for 5 minutes and electrophoresed on denaturing 10% acrylamide gels overnight. Proteins were transferred to a Hybond-ECL membrane (Amersham, Arlington Hts., IL) by standard tank transfer in Tris-glycine transfer buffer at 45 V (0.5 mA) for 3 hours. Nonspecific membrane binding was blocked by incubating the membrane in 10% nonfat dry milk in PBS-Tween overnight. After washing (3 ×), the membrane was incubated with primary antibodies (anti-TK 1:1000 and anti-GFP 1:1000, respectively, in 1% nonfat dry milk/PBS-Tween) for 1 hour at room temperature. After washing (3 ×), the membrane was incubated with horseradish peroxidase–conjugated horse antirabbit (1:4000) immunoglobulin in blocking solution for 1 hour, then washed, and developed by using the ECL reagents (Amersham).

Statistical Analyses

The mean values ± SD of cell counts and the percent differences between GCV-treated and nontreated dishes were calculated and results plotted with the software package SigmaPlot 1.02. The analysis of coexpression of HSV-1-*tk* and *gfp* genes was done with StatView 4.57 (Abacus Concepts, CA) and Kaleidagraph 3.08 (Synergy Software, CA).

Results

The tkgfp Fusion Gene was Cloned in Frame Under Control of CMV IE1 or Retroviral LTR Promoters

The expression plasmid pTKGFP bears the HSV-1-*tk* gene in-frame to the *gfp* gene under control of the CMV_{IE} promoter (Figure 1). Twenty-two base pairs (bp) are missing at the 3′ end of the open reading frame of *tk* to remove the stop codon. Eighteen base pair in-frame encoding Pro-Gly-Ser-Ile-Ala-Thr were introduced between the open reading frames for *tk* and *gfp*. HSV-1 amplicon plasmids, which feature the *tk* gene or the *gfp* gene alone under the same CMV promoter (CMV IE1; Figure 1) served as positive controls for subsequent experiments. In the retroviral vector, SFG-TKGFP, the *tkgfp* fusion gene is under control of the 5′-retroviral LTR promoter (Figure 2).

The Levels of gfp-Expression are Comparable in Cells Transduced with tkgfp and Native gfp Genes

In order to compare the levels of *gfp*-expression, plasmids pTKGFP and pHSV-GFP were transfected into rat 9L gliosarcoma cells. The levels of *gfp*-expression were assessed 1, 2, 3, and 5 days after transfection with a standard fluorescence microscope and were visually of similar inten-

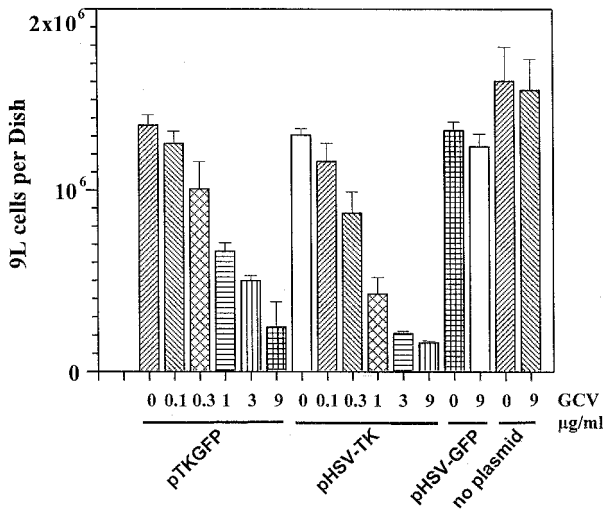

Figure 4. *Ganciclovir dose-dependent killing of proliferating rat 9L gliosarcoma cells in culture. 9L cells (600,000 per 35-mm well; DAY zero) were transfected with 1 μg DNA of each pTKGFP, pHSV-TK (positive control), pHSV-GFP, or no plasmid (negative control) by using 6 μl lipofectamine. One day after transfection, GCV (0, 0.1, 0.3, 1, 3, and 9 μg/mL medium) was added to culture plates. Four days later surviving cells were trypsinized and counted. Bars represent mean values and SD from triplicate experiment.*

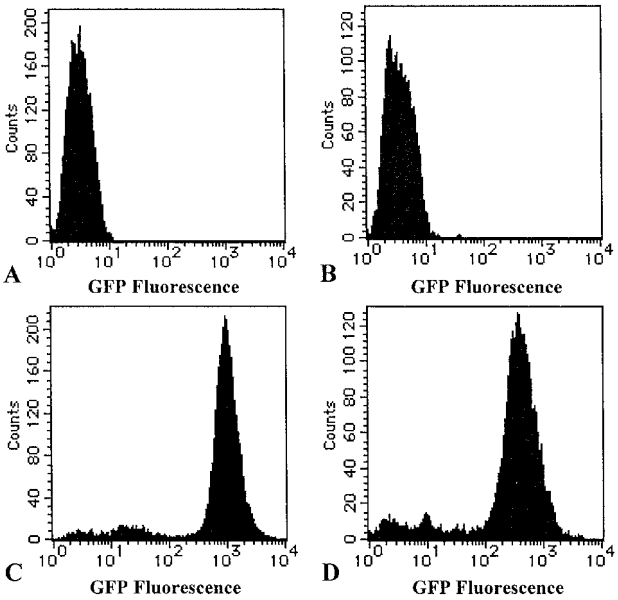

Figure 5. *FACS analysis of gfp gene expression in single cells–derived clones of RG2GFPTK+ (**C**) and W256GFPTK+ (**D**) cells. Parental RG2 cells (**A**) and W256 cells (**B**) were used as controls.*

sity in *tkgfp* and native *gfp* transfected cells (Figure 3). The data indicate that *tkgfp* transduced cells can be readily identified by fluorescent light at least to the same extent as *gfp* transduced cells. As was pointed out by Loimas et al. [36], the intracellular expression pattern of the TKGFP seems different than the native GFP. TKGFP is mostly concentrated in the nucleus, whereas the native GFP displays a predominantly cytoplasmic distribution.

GCV-Sensitivity is Similar in pTKGFP and pHSV-TK Transfected 9L Cells

In order to investigate the ability of the TKGFP fusion protein to mediate significant sensitivity towards the pro-drug ganciclovir, pTKGFP and pHSV-TK transduced 9L glioma cells (positive control) were treated with different amounts of GCV ranging from 0 to 9 μg/mL over a 5-day exposure period. After the treatment period, surviving cells were determined as a percentage of non–GCV-treated controls. In contrast to pHSV-GFP and nontransfected negative controls, the pTKGFP and pHSV-TK transfected 9L cells were both sensitive to GCV in a similar dose-dependent manner (Figure 4).

Coexpression of gfp and HSV-1-tk Genes

To investigate the spectrum of transgene coexpression that is achieved in polyclonal cell populations, we generated two series of clones to examine coexpression of both genes in the context of fusion gene expression. We obtained multiple retrovirally transduced RG2TKGFP+ and W256TKGFP+ clones with different levels of transgene expression (due to different genomic sites of retroviral integration) to explore coexpression over a broad range of transgene expression levels. The proportionality of the HSV-1-*tk* and *gfp* gene expression was assessed in culture by using the radiotracer and FACS assays, respectively. For FACS analysis of GFP, the range of fluorescence was set to include no more than 1% of corresponding control cells (Figure 5). The mean GFP fluorescence per cell was used as a measure of GFP expression in a clonal population of transduced cells. Regression analysis demonstrated a strong relationship between the levels of expression of *gfp* and HSV-1-*tk* genes in corresponding clones of retrovirally transduced RG2 glioma cells (RG2TKGFP+; Figure 6A) and in W256 carcinoma cells (W256TKGFP+; Figure 6B). Combining the RG2TKGFP+ and W256TKGFP+ data sets yields a similar relationship over a wide range of transgene expression levels (Figure 6C). Statistical analysis of the combined data also revealed that there was a significant correlation between the HSV-1-*tk* and *gfp* expression ($r = 0.894$; $P < .0001$; and $t = 2.477$; $P < .05$ based on paired *t*-test analysis). This suggests that the relationship of fusion gene–mediated coexpression is independent of tissue type.

The TKGFP Protein is Recognized by Both Anti-TK and Anti-GFP Antibodies

In order to investigate the *tkgfp* fusion gene expression at the protein level, cellular proteins of pTKGFP, pHSV-GFP,

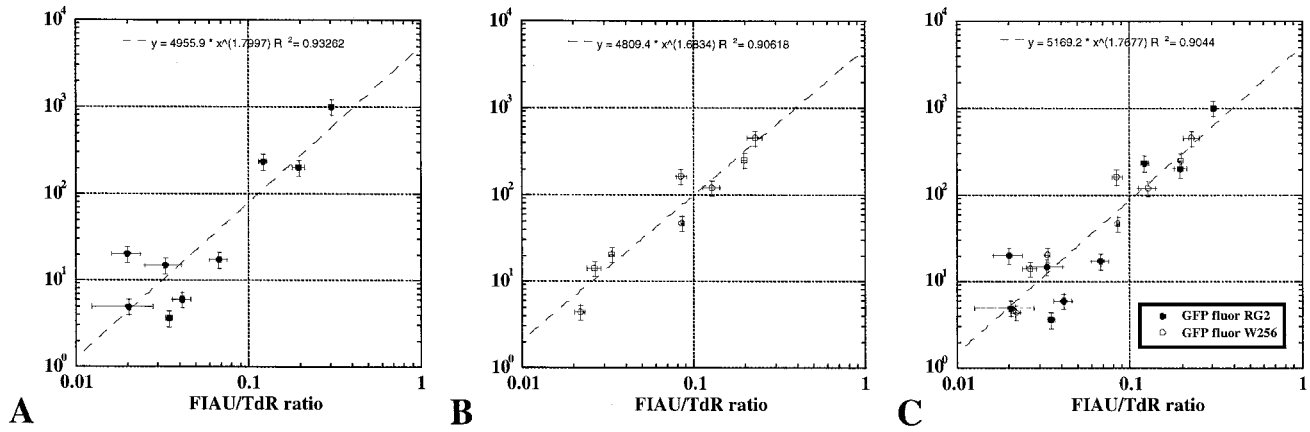

Figure 6. *Fusion-gene-mediated coexpression of the HSV-1-tk and gfp genes in culture. Gfp gene expression (mean GFP fluorscence/cell) was assessed by FACS analysis in RG2STLEO (**A**) and W256STLEO (**B**) transduced single cell clones and plotted against measurements of the HSV-1-tk gene expression (FIAU/TdR accumulation ratio). The relationship between the two measurements was defined by regression analysis. The same relationship was observed when the RG2STLEO and W256STLEO data sets were combined (**C**).*

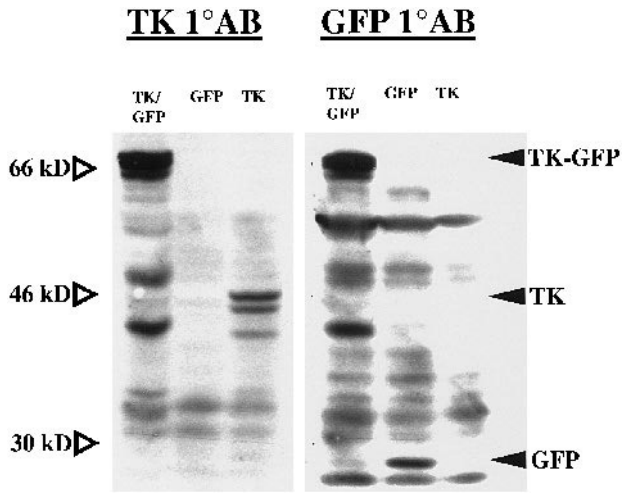

Figure 7. *Western blot analysis of the TKGFP fusion protein. 4 μg of plasmid DNA of each, pTKGFP, pHSV-GFP, and pHSV-TK, respectively, were transfected into 9L cells. Monolayers were harvested 24 hours after transfection, resuspended, lysed, boiled, and proteins electrophoresed over night. After protein transfer and blocking of unspecific binding, the membrane was incubated with primary antibodies (1°; anti-TK 1:1000 and anti-GFP 1:1000) and, after washing, with horseradish peroxidase–conjugated horse anti-rabbit (1:4000) immunoglobulin, then washed and developed. As indicated, the primary anti-TK antibody recognizes TK at approximately 46 kD (third lane from left), and the primary GFP antibody recognizes the GFP at approximately 27 kD (fifth lane from left). Both antibodies recognize the TKGFP fusion protein at greater than 66 kD (first and fourth lane from left).*

and pHSV-TK–transfected 9L cells harvested at 24 hour after transfection were resolved by SDS-PAGE and analyzed on Western blots by using antibodies to TK and GFP (Figure 7). The predominant band recognized by both the TK and the GFP antibodies is 66 kD, the appropriate size of the TKGFP fusion protein. TK and GFP proteins are, as expected, recognized only by their specific antibodies and maintain their native size (approximately 46 kD and 27 kD, respectively).

Discussion

The paradigm for noninvasive imaging of therapeutic gene expression that we propose in this report relies on proportional coexpression of a ''marker'' gene and a ''therapeutic'' gene products. Usually, the coexpression of two gene products is achieved by using a dual-promoter approach. However, on an individual cell level, as opposed to cell populations in which total expression is averaged, proportional coexpression may be compromised because of promoter interference or transcriptional repression. The proportionality of IRES-mediated coexpression of two genes is determined at the translational level and depends on protein synthetic activity and cell cycle phase of the transduced cells [45]. Strictly proportional (equimolar) coexpression of two genes under control of a single promoter can be achieved by using fusion gene approach.

To assess whether the fusion gene approach is applicable to the described imaging paradigm, we constructed the *tkgfp* fusion gene consisting of the HSV-1-*tk* marker gene (for noninvasibe imaging) and the *gfp* gene, which served as model gene for different therapeutic transgenes. Transduction of different types of tumor cells with *tkgfp* allowed the selection of transduced living cells by fluorescence microscopy and quantitation of *tkgfp* expression with FACS analysis. Expression of *tkgfp* induced sensitivity of the transduced tumor cells to GCV similar to that resulting from transduction of the cells with the same vector bearing the HSV-1-*tk* gene alone. The levels of HSV-1-*tk* gene expression in *tkgfp*- transduced clones, as measured by the FIAU accumulation, were comparable to those observed by us previoulsy in RG2 and W256 tumor cells transduced with HSV1-1-*tk* gene alone [22,23] and were within the range that is adequate for noninvasive imaging with radioiodinated FIAU and gamma camera, single photon emission computed tomography (SPECT), or PET [22–24]. Previously, we have shown that the FIAU/TdR accumulation ratio correlates highly with other independent measures of the HSV-1-*tk* gene expression: namely, ganciclovir sensitivity (IC_{50}) and concentration of HSV-1-*tk* mRNA [22].

Most importantly, we demonstrated that fusion gene approach provides a constant relationship between the expression of the HSV-1-*tk* and *gfp* genes over a wide range of expression levels. Thus, noninvasive images of HSV-1-*tk* expression should reflect distribution and level of expression of *gfp* or different therapeutic genes. Parametric images of therapeutic gene expression could be generated if the relationship between the levels of FIAU accumulation (HSV-1-*tk* expression) and functional measures of therapeutic gene expression in transduced tissues are known.

Limitations of the fusion gene–fusion protein approach is that is cannot be applied to proteins that may loose functionality as the result of fusion or will not be able to localize to the appropriate subcellular compartment. This approach cannot be applied to secretory proteins (e.g., cytokines, growth factors) because the HSV-1-*tk* marker enzyme should remain intracellular in order to catalyze the intracellular accumulation of radiolabeled FIAU. Potentially higher immunogenicity of fusion proteins may also limit their application in *in vivo* studies.

The *tkgfp* fusion gene can also be considered as therapeutic-marker gene construct, where *tk* is used as therapeutic gene and *gfp* as marker gene for *in situ* detection or direct visualization. GFP is the most widely used marker protein for direct visualization of transgene expression in living cells and transparent tissues and organisms *in vivo* [46–54]. Recently, Flotte et al. [53] implemented a videoendoscopy technique for direct visualization of *gfp*-transduced airway epithelium. Similar endoscopic technique for visualization of *tkgfp* fusion gene expression could be useful for gene therapy directed to the gastrointestinal tract by using the HSV-1-*tk*/GCV paradigm. Therefore, *tkgfp* fusion gene could be used as a dual-marker gene for noninvasive imaging, direct endoscopic visualization, and *in situ* detection with fluorescence microscopy.

From a vector design prospective, the *tkgfp* fusion gene has several positive features. A relatively small coding region of *tk* (1.2 kb) and *gfp* (750 bp) results in a *tkgfp* fusion

gene that is less than 2 kb in size and allows for easy incorporation into virus vectors with small transgene capacities (e.g., adeno-associated virus vector). The transgene capacity of adeno-associated virus vectors is less than 5 kb, which makes cloning of two transgenes (therapeutic and selection gene) within the same vector difficult. The GFP-fluorescence is not dependent on substrates or cofactors, which enables direct detection of transgene expression in living cells. Therefore, the need for time-consuming immunocytochemistry and histochemistry for the detection of transduced cells can be avoided. This is particularly important because of the lack of a readily available antibody for HSV-1-*tk*. Furthermore, GFP-expressing cells can be rapidly isolated from a pool of transduced cells by FACS. This avoids time-consuming drug selection and allows for fractionation of cells with respect to the level of GFP expression and, in turn, the level of *tk* expression.

Summary

The proportionality of coexpression of two genes in fusion, the HSV-1-*tk* and *gfp*, was demonstrated over the wide range of expression levels. Fusion gene approach should be applicable to the paradigm of monitoring therapeutic transgene expression by noninvasive imaging of marker gene coexpression. Noninvasive imaging of transgene expression should facilitate assessment of the efficacy and target specificity of novel gene delivery vectors by defining the distribution, level, and temporal profile of expression of different therapeutic genes *in vivo*. It would contribute significantly to further development of vector technology and gene therapy in general.

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